IS THE K PERMEABILITY OF THE RESTING MEMBRANE CONTROLLED BY THE EXCITABLE K CHANNEL?

DONALD C. CHANG
Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas 77030; and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

ABSTRACT To test whether or not the potassium permeability of the resting membrane is controlled by the excitable K channels (delayed rectifier), we examined changes in the Na and K permeability ratio, P_{Na}/P_K, of the squid axon before and after the excitable K channels were blocked. The blockage of the K channels was accomplished by three independent methods: (*a*) internal application of tetraethylammonium, (*b*) internal application of 4-aminopyridine plus Cs, and (*c*) prolong internal perfusion of NaF solution. The permeability ratio was determined using two different methods: the conventional electrophysiological method and a new method based on the measurements of the hyperpolarizing effect of Na removal. We found that blocking the K channels did not cause a proportional decrease in the K permeability of the resting membrane, suggesting that the semipermeable property of the resting membrane is not determined by the excitable K channels.

INTRODUCTION

It is well known that the resting membrane of the nerve cell has a higher permeability to the K^+ ions than to Na^+ ions. However, it is not clear whether this selectivity is determined by the number of excitable Na and K channels that stay open in the resting state, or if there exists a different kind of "resting pathway" in the membrane that is selectively permeable to the K^+ ions (Freeman, 1971; Narahashi et al., 1971; Yamagishi and Grundfest, 1971; Chang and Liu, 1985). During early development of the ionic theory, no distinction was made between mechanisms responsible for the resting permeability and those responsible for the excitable conductance. For example, Baker et al. (1962) proposed that the permeability ratio (P_{Na}/P_K) of the squid giant axon at various resting potential is determined by the potassium and sodium conductances calculated from the equations of Hodgkin and Huxley (1952*b*). This proposal hypothesized that excitable channels provided the major pathway for the resting currents which determine the resting potential. A similar view was also expressed in a number of later studies (Freeman, 1971; Narahashi et al., 1971; Edwards and Vyskocil, 1984).

Although the hypothesis of Baker et al. (1962) is a reasonable and attractive one, it has not been vigorously tested experimentally. Recently, Chang and Liu (1983) showed that blocking the excitable Na channels by tetrodotoxin (TTX) had much less effect on the resting potential than the removal of external Na. This observation suggested that in the squid axon the excitable Na channels were not the major pathway for the resting Na current. Since the majority of resting currents are carried by K ions, the more important question is whether the resting K current, which to a large extent determines the resting potential, passes through the excitable K channels. Unlike the case of Na channels, many nonexcitable cells that have negative resting potential are known to have excitable K channels (Sheehy et al., 1986; Lee et al., 1986; Rae, 1985; Sauve et al., 1984). Furthermore, since the voltage-dependent K conductance does not inactivate quickly like the Na conductance, it is theoretically possible that the excitable K channel could contribute more significantly to the steady state permeability.

In this study we used the squid giant axon as a biological model to examine if the resting K current passes mainly through the excitable K channels. Our objective was to determine whether a proportional decrease in K permeability occurs after abolition of K channel in the resting membrane. Since no pharmacological treatment is totally selective in blocking the excitable K channel, three different methods were used. The effectiveness of each treatment was measured by a voltage-clamp technique. The changes of P_{Na}/P_K of the resting membrane during each treatment were determined using two electrophysiological methods.

METHODS AND MATERIALS

The giant axons used in this study were dissected from fresh, live squids (*Loligo pealei*) provided by the Marine Biological Laboratory, Woods Hole, MA. The resting potential of the axons was measured using an axial internal electrode as described previously (Chang, 1983). Junction potentials of the electrode system were corrected by using an experimental design similar to that of Baker et al. (1964). (Between the different solutions used in this experiment, the junction potential varied within 1 mV.) Our method of internal perfusion was a modification of the cannulation method developed by Tasaki (Tasaki et al., 1965).

The normal external solution used in this study was K-free artificial seawater (ASW) which consisted of 430 mM NaCl, 20 mM $CaCl_2$, 50 mM $MgCl_2$, and 2.5 mM HEPES buffer. Approximately 2 mM NaOH was added to adjust the pH of the ASW to 7.9. The Na-free ASW was similar to the K-free ASW in composition except that NaCl was replaced by choline chloride. The normal internal perfusion solution (IPS) consisted of 360 mM KF, 50 mM Na-glutamate, and 40 mM K-phosphate buffer. The pH of the IPS was adjusted to 7.3. To make the IPS isotonic to the ASW, 200 mM of sucrose was also added. (For the IPS which contained an additional 100 mM of CsCl, the concentration of sucrose was reduced to 66 mM). The composition of our NaF and KF solutions was similar to that used by Chandler and Meves (1970). The experiments were done at 6° C.

The activity of Na^+ and K^+ in our internal and external solutions were determined using two different methods. The first one was to calculate the effective activity coefficient of an ion in a given solution based on the data tabulated by Robinson and Stokes (1959). The second one was to measure the ionic activity using an ion-selective electrode. Both methods gave similar results. For example, the Na activities in our normal internal and external solutions calculated by the first method were 289 mM and 29.7 mM, respectively. The same activities determined experimentally by a sodium-selective electrode (Model 94-11A, Orion Research Inc., Cambridge, MA) were 286 mM and 28.8 mM, respectively. The K activities of our normal IPS and the IPS with 100 mM Cs were calculated to be 253 mM and 250 mM, respectively.

RESULTS

Measurement of P_{Na}/P_K Ratio

We used two different methods to determine the permeability ratio P_{Na}/P_K of the resting membrane. The first method was a conventional one. That is, we measured the resting potential of the squid axon and then applied the Goldman-Hodgkin-Katz (GHK) equation (Hodgkin, 1958) to calculate the P_{Na}/P_K ratio. In general, the resting potential is dominated by the gradient of K^+ across the membrane. In order to make the potential more sensitive to the P_{Na}/P_K ratio, we removed the K^+ ions from the external solution (i.e., using K-free ASW) during the potential measurement. The resulting P_{Na}/P_K determined by this method is summarized in Table I under the column labelled "P_{Na}/P_K by GHK." (Note that here the permeability ratio was calculated based on ionic activities rather than concentrations.)

Our second method of determining P_{Na}/P_K utilized the fact that as external Na^+ ions are removed from the ASW, the axon hyperpolarizes. The magnitude of this hyperpolarization is related to the value of P_{Na}/P_K (Freeman, 1971; Chang and Liu, 1985). It has been shown that the resting potential of a squid axon does not always follow the GHK equation (Chang, 1983). Instead, the potential is better described by a modified constant field equation (Chang, 1983), i.e.,

$$V = \frac{RT}{F} \ln \left\{ \frac{[K]_o + b[Na]_o}{[K]_i + b[Na]_i} + c \right\}, \quad (1)$$

where R, T, and F are the universal gas constant, temperature, and the Faraday constant, respectively; b is the P_{Na}/P_K ratio, and c is a constant that depends on the concentrations of divalent ions and the diffusive properties of the periaxonal space. When the axon is bathed in K-free ASW, one can show from Eq. 1 that

$$b = \frac{[K]_i}{[Na]_o} e^{FV/RT} (1 - e^{F\Delta V/RT}), \quad (2)$$

where ΔV is the potential change when the external solution was changed from normal K-free ASW to K-free, Na-free ASW. ($[Na]_o$ is the original Na concentration before the solution change.)

We measured ΔV in 26 squid axons under various chemical treatments. Samples of some of these measurements are shown in Fig. 1. ΔV in general was less than 5 mV, indicating that in all cases, the resting Na current must be relatively small. The average values of ΔV under different chemical treatments are summarized in Table I. From the values of V and ΔV, P_{Na}/P_K was calculated using Eq. 2. The results are tabulated in Table I in the column labelled "P_{Na}/P_K by Na^+-removal."

Blocking of K Channels

To examine how the membrane permeability responds to changes in the excitable K channel, we used three different methods to abolish (or block) the K channel. The first

TABLE I
PERMEABILITY RATIO BETWEEN Na^+ AND K^+ OF THE RESTING MEMBRANE OF THE SQUID AXON

Treatment	% of K channel blocked	Resting potential	Effect of Na^+ removal	P_{Na}/P_K	
				by GHK	by Na^+ removal
		V(mV)	ΔV(mV)		
Control	0	−69.2±1.6 (24)	−4.3±1.2 (25)	0.049	0.0080
TEA	97.6	−68.4±1.3 (5)	−5.3±1.0 (8)	0.050	0.0099
K^+-free	91.7	−61.0±1.7 (4)	−6.3±1.2 (6)	0.069	0.0159
4-AP + Cs^+/TTX	99.2	−57.8±4.5 (4)	+1.1±0.7 (9)	0.078	—
TTX	0	−68.6±2.3 (10)	−3.8±1.1 (15)	0.050	0.0074

The percentage of K channel blocked was determined from voltage-clamp records similar to those shown in Fig. 2. The values of P_{Na}/P_K by GHK were calculated from the resting potential using the GHK equation. The values of P_{Na}/P_K by Na^+ removal were calculated from V and ΔV using Eq. 2. For those axons treated with 4-AP + Cs^+ and TTX, the resting potential did not hyperpolarize when external Na^+ was removed. Eq. 2 thus was not applicable. Ionic activities instead of ionic concentrations were used in the calculation of P_{Na}/P_K.

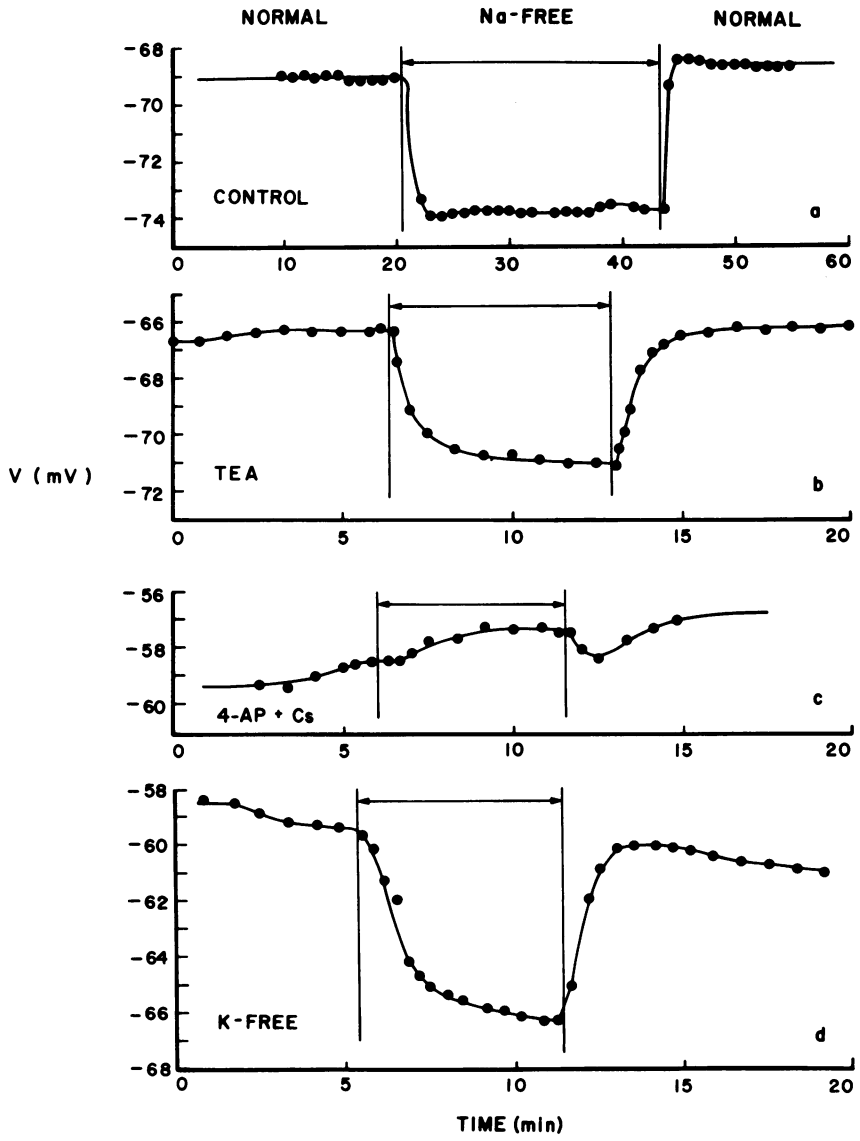

FIGURE 1 Sample data showing the changes of the resting potential in response to the removal of external Na^+. The axon was first bathed in K-free ASW which contains 432 mM Na^+. At the time period marked by the arrow, the external solution was changed to K-free Na-free ASW. After that period, the axon was bathed in K-free ASW again. (*a*) Control. (*b*) 20 mM TEA was applied inside the axon throughout the measurement. (*c*) 2 mM 4-AP + 100 mM Cs^+ were applied inside the axon. (*d*) Same as control except that the axon was previously perfused internally with NaF solution for 30 min.

method was internal application of 20 mM tetraethylammonium (TEA), which is known to block the delayed rectifier (Armstrong and Binstock, 1965). In addition to determining the P_{Na}/P_K, we also used the voltage-clamp method to measure the quantitative effects of 20 mM TEA on the ionic conductances g_K and g_{Na}. A sample record of such a measurement is shown in Fig. 2. Fig. 2 *a* shows the voltage-clamp current traces of a control axon (no treatment) when the axon was depolarized from −70 mV to +70, +50, +30, +10, −10, −30 mV (from top to bottom). Fig. 2 *b* shows current traces from an experiment with identical voltage steps when 20 mM TEA was applied inside the axon. It is evident that most of the outward currents were suppressed. By using an I-V plot, one can determine the potassium conductance from the delayed current at the reversal potential of Na^+ (in this case +52 mV). A comparison of the K conductance before and after the TEA treatment indicates that under our experimental conditions, TEA can reduce the g_K by 30-fold.

One may notice from Figs. 2 *a* and *b* that the TEA treatment also reduced the sodium conductance by 20%. This effect was reproducible in many axons and could not be eliminated by repurifying TEA. A similar finding was also observed in other laboratories although it has not been formally reported (Narahashi, private communication).

The second method of reducing g_K was to apply 2 mM 4-aminopyridine (4-AP) and 100 mM Cs^+ internally. Under this treatment, the delayed K current disappeared

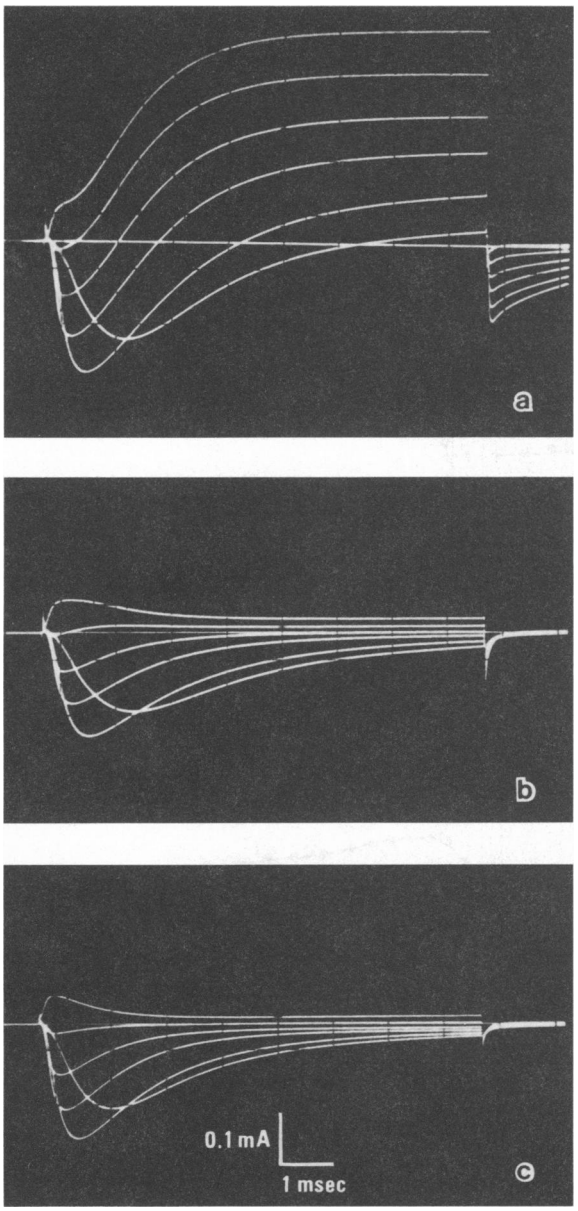

FIGURE 2 Membrane current of the axon under voltage-clamp is plotted as a function of time. Traces (from *top* to *bottom*) represent currents recorded when the potential of the axon was changed from −70 mV to 70, 50, 30, 10, −10, −30 mV. (*a*) Control. (*b*) 20 mM TEA was applied inside. (*c*) 2 mM 4-AP + 100 mM Cs^+ was applied inside.

almost completely from the voltage-clamp record (Fig. 2 *c*). We estimated that over 99% of the K channels were blocked by a combination of 4-AP and Cs^+. This treatment, however, had one complication. When the axon was bathed in normal Na ASW, the 4-AP + Cs^+–treated axon always gave spontaneous firing and thus it was difficult to measure its resting potential. To suppress this spontaneous firing, we applied 0.4 μM TTX in the ASW when the resting potential was measured. It is known that TTX has little effect on the resting potential of the squid axon (Chang and Liu, 1985). (Also see Table I.)

Our third method of abolishing the excitable K channel did not involve any chemical additive. The axon was perfused internally and externally with K-free solutions for approximately 1 h before it was returned to the normal internal and external solutions. It has been shown by Chandler and Meves (1970) and Almers and Armstrong (1980) that prolonged internal perfusion with NaF solution (with no external K^+ ion) can remove the excitable K conductance irreversibly. Our voltage-clamp measurement confirmed these findings. Fig. 3 shows the effect of K-free treatment. In Fig. 3 *a*, current traces of a voltage-clamped axon before the K-free treatment (i.e., control) are recorded. The internal solution was an IPS with 300 mM K^+ and no Na^+. The external solution was normal ASW. Fig. 2 *b* shows current traces of the same axon with identical internal and external solutions after the axon had

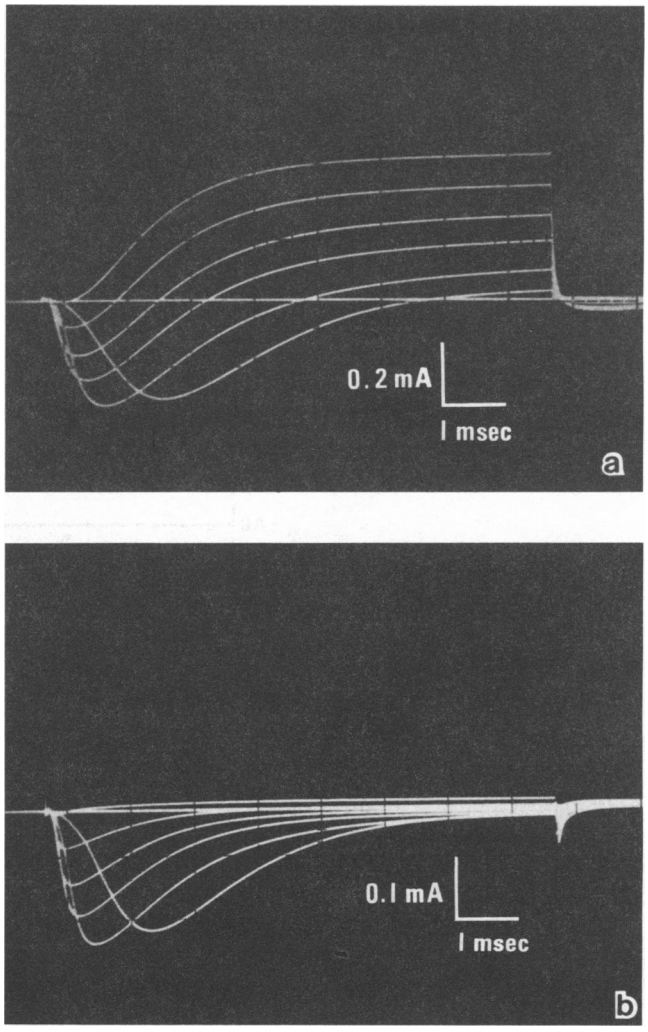

FIGURE 3 Sample record of current traces of an axon under voltage-clamp before (*a*) and after (*b*) the axon was internally perfused with NaF solution. Figs. 3, *a* and *b* were recorded under identical chemical environments. That is, the axon was perfused with 300 mM KF IPS inside and normal ASW outside. The length of the K-free treatment was 32 min. The axon was depolarized from a potential of −70 mV to 70, 50, 30, 10, −10, −30 mV (corresponding to current traces from *top* to *bottom*).

been exposed to NaF IPS and K-free ASW for 32 min. The outward K current was almost completely abolished.

Comparison between g_K and P_K

Table I summarizes the results of the voltage-clamp measurements and the estimated values of the P_{Na}/P_K ratio under different treatments. It is apparent that all three treatments used here are effective in blocking the excitable K current. Under our experimental conditions, TEA, 4-AP + Cs, and the K^+-free treatment each would block 98%, 99%, and 92% of the K channels in the squid axon, respectively. The P_{Na}/P_K ratio, on the other hand, did not seem to be significantly affected by the blocking of K channels. No matter whether the conventional GHK method or the method of Na^+ removal was used, the value of P_{Na}/P_K under different chemical treatments varied by less than a factor of two. Apparently, the permeability ratio of the resting membrane is not correlated with the blocking of the excitable K channel.

DISCUSSION

The results of our measurements suggest that most of the resting K current passes through pathways different from the excitable K channels. For example, when over 97% of K channels were blocked by internal application of TEA, the P_{Na}/P_K ratio remained almost unchanged from the control value. If the K channels were the major pathways for the resting K current, the TEA treatment should increase the P_{Na}/P_K value by 30-fold. The axons under the 4-AP + Cs^+/TTX treatments also demonstrated that the semipermeable property of the resting membrane is not determined by the excitable ionic channels. Here, despite the fact that all of the K channels were essentially blocked by 4-AP + Cs^+ and the Na channels were completely blocked by TTX, the axons still maintained a resting potential of -58 mV, which is not too much different from that of a normal axon.

The results of our experiments are consistent with an early study by Tasaki and Hagiwara (1957) who showed qualitatively that TEA does not affect the resting membrane potential. In this study, we also demonstrated that the lack of correlation between the permeability ratio and the state of the K channels cannot be attributed to an interfering effect due to added pharmacological agents, since in the case of the prolonged K-free treatment, the permeability ratio measurements were done before and after abolishing the K conductance using identical IPS and ASW with no drugs added. Yet, P_{Na}/P_K changed by less than a factor of two when over 90% of K channels had been removed.

One may wonder if the resting potential of the axon was affected by a chloride gradient, and, thus blocking the K channel, may fail to depolarize the axon. However, this was found not to be the case. It has been shown in several laboratories that chloride ions, either internally or externally, have no significant effect on the resting potential of the squid giant axon (Baker et al., 1962; Tasaki et al., 1965; Chang, 1983).

We cannot rule out the possibility that the sodium permeability, P_{Na}, may also vary with the P_K under the chemical treatments. Suppose that blocking the K channels causes a proportional decrease not only in P_K but also in P_{Na}. Then the P_{Na}/P_K ratio would be relatively constant under the K channel block. This, however, is not likely the case. Since we used three separate treatments to block the K channels and none of them caused a significant increase in the P_{Na}/P_K value, one would have difficulty in explaining why blocking the K channels would decrease the P_{Na} proportionally in all cases.

In some excitable cells there is more than one type of excitable K channel. For the squid giant axon, the delayed rectifier is the predominant type of K channel. Other types of K channel, such as the inward rectifier that is known to play an important role in the maintenance of the resting potential in certain cells, were not found in the squid axon (Katz, 1949).

Our finding that the P_K of the resting membrane of the squid axon is not controlled by excitable K channels suggests that the cell membrane may contain two types of macromolecular mechanisms, one of which is responsible for maintaining the resting potential, while the other generates the excitation signal. Since most nonexcitable cells also maintain a negative resting potential, the first type of mechanism apparently is shared by both excitable and nonexcitable cells. At this point very little is known about the molecular nature of these resting pathways. These pathways must be some sort of membrane proteins through which ions can transport passively, but they need not be channels. Since the ion transport rate at the resting state is very slow, one cannot rule out the possibility that the resting pathways could be carriers.

Many electrophysiologists, especially those working with voltage-clamping, often designate the part of currents which do not pass through the excitable channels as "leakage current." Such terminology may not be entirely appropriate and needs to be clarified. The "leakage current" originally described by Hodgkin and Huxley (1952*a*) consisted mainly of currents carried by Cl^- or other divalent ions, and currents going through cut-branches or the active transport system. It did not include the resting currents carried by K^+ and Na^+ that give rise to the normal resting potential. Our finding in this study suggests that these resting K (or Na) currents pass through separate types of pathways that are neither the excitable channels nor the "leakage pathway" as described by Hodgkin and Huxley (1952*a*).

In conclusion, this study suggests the existence of specific pathways at the axon membrane that have a high selectivity for K^+ over Na^+ or anions. These resting pathways apparently are different from the excitable K channels (delayed rectifier) because they have different

pharmacological properties. These resting pathways are not only resistant to TEA, 4-AP and internal Cs^+, they can also survive prolonged treatment of NaF solution.

This work was supported in part by a grant from National Science Foundation (BNS-84-06932) and an ONR contract (N00014-85-K-0424).

I thank Drs. Tom Reese, C. N. Fong, Raymond Glantz, and R. L. Vick for their comments. I also thank Z. Y. Zhao and J. Liu for their technical assistance and Bonnie Lee for her secretarial assistance.

Received for publication 27 June 1986 and in final form 4 August 1986.

REFERENCES

Almers, W., and C. M. Armstrong. 1980. Survival of K^+ permeability and gating currents in squid axon perfused with K^+ free media. *J. Gen. Physiol.* 75:61–78.

Armstrong, C. M., and L. Binstock. 1965. Anomalous rectification in the squid giant axon injected with tetraethyl-ammonium chloride. *J. Gen. Physiol.* 48:859–872.

Baker, P. F., A. L. Hodgkin, and H. Meves. 1964. The effect of diluting the internal solution on the electrical properties of a perfused giant axon. *J. Physiol. (Lond.).* 170:541–560.

Baker, P. F., A. L. Hodgkin, and T. I. Shaw. 1962. The effects of changes in internal ionic concentrations on the electrical properties of perfused giant axons. *J. Physiol. (Lond.).* 164:355–374.

Chandler, W. K., and H. Meves. 1970. Sodium and potassium currents in squid axons perfused with fluoride solutions. *J. Physiol. (Lond.).* 211:623–652.

Chang, D. C. 1983. Dependence of cellular potential on ionic concentrations: Data supporting a modification of the constant field equation. *Biophys. J.* 43:149-156.

Chang, D. C., and J. Liu. 1985. A comparative study of the effects of tetrodotoxin and the removal of external Na^+ on the resting potential: evidence of separate pathways for the resting and excitable Na currents in squid axon. *Cell. Mol. Neurobiol.* 5:311–320.

Edwards, C., and F. Vyskocil. 1984. The effects of the replacement of K^+ by Tl^+, Rb^+, and NH_4^+ on the muscle membrane potential. *Gen. Physiol. Biophys.* 3:259–264.

Freeman, A. R. 1971. Electrophysiological activity of tetrodotoxin on the resting membrane of the squid giant axon. *Comp. Biochem. Physiol.* 40A:71–82.

Hodgkin, A. L. 1958. Ionic movements and electrical activity in giant nerve fibres. *Proc. Royal Soc. Lond. B Biol. Sci.* 148:1–37.

Hodgkin, A. L., and A. F. Huxley. 1952*a*. The components of membrane conductance in the giant axon of Loligo. *J. Physiol. (Lond.).* 116:473–496.

Hodgkin, A. L., and A. F. Huxley. 1952*b*. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.).* 117:500–544.

Hodgkin, A. L., and B. Katz. 1949. The effect of sodium ions on the electrical activity of the giant axon of squid. *J. Physiol. (Lond.).* 108:37–77.

Katz, B. 1949. Les constantes electriques de la membrane du muscle. *Arch. Sci. Physiol.* 2:285–299.

Lee, S., D. Krause, and C. Deutsch. 1985. Increased voltage-gated K conductance in T-lymphocytes stimulated with phorbol ester. *Biophys. J.* 47:147*a*. (Abstr.)

Narahashi, T., E. X. Albuquerque, and T. Deguchi. 1971. Effects of batrachotoxin on membrane potential and conductance of squid giant axons. *J. Gen. Physiol.* 58:54–70.

Rae, J. L. 1985. Blockers of Ca-activated potassium channels from lens epithelium. *Biophys. J.* 47:136*a*. (Abstr.)

Robinson, R. A., and R. H. Stokes. 1959. Electrolyte Solutions. Butterworths, London. 803 pp.

Sauve, R., G. Bedfer, and R. Roy. 1984. Single Ca^{++} dependent K currents in HeLa cancer cells. *Biophys. J.* 45:66–68.

Sheehy, P. A., E. A. Grimm, and J. L. Barker. 1986. Late outward current in cultured lymphokine-activated human lymphocytes. *Biophys. J.* 49:165*a*. (Abstr.)

Tasaki, I., and S. Hagiwara. 1957. Demonstration of two stable potential states in the squid giant axon under tetraethylammonium chloride. *J. Gen. Physiol.* 40:859–885.

Tasaki, I., I. Singer, and T. Takenaka. 1965. Effect of internal and external ionic environment on excitability of squid giant axon. *J. Gen. Physiol.* 48:1095–1123.

Yamagishi, S., and H. Grundfest. 1971. Contributions of various ions to the resting and action potentials of crayfish medial giant axons. *J. Membr. Biol.* 5:345–365.